WEAK PARTHENOGENETIC RACES OF HYDATINA SENTA SUBJECTED TO A VARIED ENVIRONMENT.

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Some years ago Weismann maintained that the unicellular organisms were not subject to natural death but were immortal. No individual died from old age but in reproduction went to form the offspring. Later Maupas made some observations upon pedigreed cultures of certain of the protozoa and found that, although the individuals did not develop senile decay, nevertheless, the race did go gradually into senile decay and died out if no conjugation was allowed. Later Calkins, Woodruff, Gregory, and others confirmed Maupas's observations but, in addition, Calkins and Woodruff have found that when the races were very weak and near the point of death they could be artificially stimulated and restored to their former vigor by various substances in the food solution. After which they were able to reproduce for many more generations before becoming weak again and then could be restimulated again. However, there always came a time when nothing would reinvigorate the races and they consequently died out. Recently Woodruff has shown that certain races of paramœcia never become weak provided the environment is more or less varied.

In recent papers Whitney has shown that the rotifer, *Hydatina senta*, can be propagated parthenogenetically for several hundred generations but each race gradually becomes weaker and weaker and finally dies out. However, when they are in this weak condition the races may be restored to the normal degree of vigor by cross-breeding. Close-breeding within each race only slightly restores their vigor.

At the time many experiments were made by changing the environment in order to determine whether any external influence would restore these weak races to their normal vigor as had been done in the weak races of protozoa.

The two weak races, *A* and *B*, which have been fully described in a former paper were used in these experiments. The criterion of weakness was the rate of reproduction. Races *A* and *B* in the spring of 1911 were allowed to produce close-fertilized eggs in the 370th and the 380th parthenogenetic generations respectively. From these close-fertilized eggs females developed which reproduced parthenogenetically for a time but by the end of the summer they had been allowed to close-breed three or four times. At this period most of the experiments described in this paper were undertaken.

Ever since the two races were started in the fall and winter of 1908 and 1909 they have been subjected to a very constant environment. They have been kept at room temperature ranging from 18° C. to 22° C. and always have been in a food solution of horse manure. During the last sixteen months of their parthenogenetic propagation they were even fed upon a pure culture of the flagellate, *Polytoma*, which was reared in a horse manure solution. A certain quantity of this horse manure solution containing the protozoa was added to a certain amount of tap water and placed in syracuse watch glasses thus making the amount and concentration of the food culture water in which the rotifers lived practically constant.

Different Food Materials.

In order to cause a great variation in the food factor and also of the chemicals in the water food cultures were prepared from the feces of various herbivorous, carnivorous and omnivorous animals. These food cultures were made in battery jars and inoculated with a miscellaneous lot of protozoa from several small fresh water ponds. The rotifers were placed in these large jars and allowed to live freely from 9 to 19 days and then were transferred to other food jars which contained feces of a different animal. In this manner a great variation of protozoa and of chemical substances in the water were obtained. These experiments extended through about three months. Table I. shows the data obtained at the end of the experiments which demonstrate that no reinvigoration had taken place in either of the two weak races.

Table I.

Table showing the comparative reproduction rates of various weak races after they had been subjected to culture waters, from 9–19 days, which were made from the following feces: horse, Sept. 18–27; guinea-pig, Sept. 27 to Oct. 9; man, Oct. 9–23; dog, Oct. 23 to Nov. 10; sheep, Nov. 10–25; hen, Nov. 25 to Dec. 14; horse, Dec. 14——. These comparative reproduction rates show that the general vigor of the weak races was not restored to that of the normal race (control) by the varied environment.

Experiment.	Time, 1911.	Control. Race *A* Between 470–500 Parthenogenetic Generations.			*A* 3d. (Race *A* Close-fertilized 3 Successive Times After the 370th Parthenogenetic Generation, March–September.)			*B* 4th. (Race *B* Close-fertilized 4 Successive Times After the 380th Parthenogenetic Generation, March–September.)			Control. Race *C* at the 390th Parthenogenetic Generation.			Control. Parthenogenetic Race *F* Developed from a Wild Fertilized Egg November 22, 1911.			Generations.
		Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	
1	Eve. 12–14 Eve. 12–17	6	1	0.16	2	1	0.5	4	2	0.5	8	44	5.5	8	87	10.87	1
	Eve. 12–18 Eve. 12–21	9	6	0.66	7	29	4.14	8	11	1.37	8	42	5.25	9	97	10.77	2
	Eve. 12–21 Eve. 12–24	4	4	1	10	22	2.2	9	17	1.88	10	49	4.9	8	69	8.62	3
2	Eve. 12–16 Eve. 12–19	5	5	1	1	4	4	1	2	2	5	44	8.8	3	42	14	1
	Eve. 12–19 Eve. 12–22	8	3	0.37	9	25	2.77	9	5	0.55	8	19	2.37	9	51	5.66	2
	Eve. 12–22 Eve. 12–25	10	3	0.3	10	25	2.5	9	10	1.11	10	32	3.2	8	56	7	3
	Summary	42	22	0.52	39	106	2.71	40	47	1.17	49	230	4.69	45	402	8.93	

Rest.

It is sometimes suggested that a period of inactivity will stimulate weak organisms. Therefore both races were kept at 3° C. to 7° C. for about three weeks in a rich food culture and then were placed at room temperature but failed to show any increase in the rate of reproduction. Some close-fertilized eggs of race *B* were kept in water at room temperature for about a year and then allowed to hatch. Table II. shows that this long period of rest produced no stimulation upon the race.

Table II.

Experiment.	Time, 1912.	Close-fertilized Eggs of Race *B* Kept in Water About a Year at Room Temperature.			Control.		
		Young Sisters whose Mother Developed from a Wet Egg.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.
1	Eve. 2–17 Eve. 2–20	5	17	3.4	10	134	13.4
2	Eve. 2–17 Eve. 2–20	5	38	7.6	10	134	13.4
3	Eve. 2–17 Eve. 2–20	5	32	6.4	10	134	13.4
4	Eve. 2–17 Eve. 2–20	4	18	4.5	10	134	13.4
5	Eve. 2–17 Eve. 2–20	5	26	5.2	10	134	13.4
6	Eve. 2–17 Eve. 2–20	3	5	1.66	10	134	13.4
7	Eve. 2–19 Eve. 2–22	4	0	0	4	48	12
8	Eve. 2–19 Eve. 2–22	5	5	1	4	48	12
9	Eve. 2–19 Eve. 2–22	5	31	6.2	4	48	12
10	Eve. 2–19 Eve. 2–22	4	5	1.25	4	48	12
	Summary	45	177	3.93	14	182	13

Close-fertilized eggs of race *A* were dried and kept at room temperature for about eight months. Table III. shows the negative results obtained.

Temperature.

Of course, temperature has much to do with the state of activity of the particles of matter. In order to further test the

hypothesis of inactivity some fertilized eggs of race *A* while still in water were kept at $-70°$ C. for twenty-four hours. Other fertilized eggs were dried and kept in liquid air[1] at a temperature of about $-191°$ C. for four days. Both lots of these eggs were

TABLE III.

Experiment.	Time, 1912.	Close-fertilized Eggs of Race *A* Dried 8 Months at Room Temperature.			Control.		
		Young Sisters whose Mother Developed from a Dried Egg.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.
1	Eve. 2–21 Eve. 2–24	4	42	10.5	6	104	17.33
2	Eve. 2–24 Eve. 2–27	4	49	12.25	5	88	17.6
3	Eve. 2–25 Eve. 2–28	3	29	9.66	8	163	20.37
4	Eve. 2–25 Eve. 2–28	5	59	11.8	8	163	20.37
5	Eve. 2–25 Eve. 2–28	5	64	12.8	8	163	20.37
6	Eve. 2–26 Eve. 2–29	5	22	4.4	7	90	12.85
7	Eve. 2–26 Eve. 2–29	3	11	3.66	7	90	12.85
8	Eve. 2–26 Eve. 2–29	5	17	3.2	7	90	12.85
9	Eve. 2–26 Eve. 2–29	4	34	8.5	7	90	12.85
10	Eve. 2–26 Eve. 2–29	4	27	6.75	7	90	12.85
11	Eve. 2–27 Eve. 3–1	5	40	8	2	27	13.5
12	Eve. 2–27 Eve. 3–1	4	31	7.75	2	27	13.5
	Summary	51	425	8.33	28	472	16.85

hatched and the rate of reproduction of the developing females compared with that of the control. Tables IV. and V. show that no reinvigoration had taken place.

Some dried fertilized eggs were placed at a high temperature of $+100°$ C. for six hours. Table VI. shows that race *A* was not stimulated by this high temperature.

[1] I am greatly indebted to Professor W. P. Bradley, of the department of chemistry of Wesleyan University, for his kindness in personally manufacturing and donating the numerous liters of liquid air which were used in these experiments.

TABLE IV.

Experiment.	Time, 1912.	Wet Close-fertilized Eggs of Race *A*, Kept at $-70°$ C. for 24 Hrs.			Control.		
		Young Sisters whose Mother Developed from a Fertilized Egg.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.
1	Eve. 2–14, Eve. 2–17	5	29	5.8	8	80	10
2	Eve. 2–14, Eve. 2–17	5	28	5.6	8	80	10
3	Eve. 2–14, Eve. 2–17	3	16	5.33	8	80	10
4	Eve. 2–14, Eve. 2–17	5	36	7.2	8	80	10
	Summary	18	109	6.05	8	80	10

TABLE V.

Experiment.	Time, 1912.	Dried Close-fertilized Eggs of Race *A*, Kept in Liquid Air at About $-191°$ C. for Four Days.			Control.		
		Young Sisters Whose Mother Developed from a Dried Egg.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.
1	Eve. 3–14, Eve. 3–17	3	29	9.66	5	92	18.4
2	Eve. 3–14, Eve. 3–17	2	21	10.5	5	92	18.4
3	Eve. 3–14, Eve. 3–17	4	44	11	5	92	18.4
4	Eve. 3–15, P. M. 3–18	2	11	5.5	6	73	12.16
5	Eve. 3–15, P. M. 3–18	2	7	3.5	6	73	12.16
6	Eve. 3–15, P. M. 3–18	1	7	7	6	73	12.16
7	A. M. 3–17, Eve. 3–19	3	6	2	4	32	8
8	A. M. 3–17, Eve. 3–19	3	9	3	4	32	8
9	A. M. 3–17, Eve. 3–19	4	23	5.6	4	32	8
10	A. M. 3–17, Eve. 3–19	2	4	2	4	32	8
11	A. M. 3–17, Eve. 3–19	3	13	4.25	4	32	8
	Summary	29	174	6	15	197	13.13

TABLE VI.

Experiment.	Time, 1912.	Dried Fertilized Eggs of Race *A*, Kept at + 100° C. for 6 Hrs.			Control.		
		Young Sisters whose Mother Developed from a Dried Egg.	Their Offspring of Daughter-females.	Average Number of Daughter-females.	Young Females Isolated.	Their Offspring of Daughter-females.	Average Number of Daughter-females.
1	Eve. 3–4 / Eve. 3–7	2	11	5.5	4	69	17.25
2	Eve. 3–4 / Eve. 3–7	4	39	9.75	4	69	17.25
3	Eve. 3–4 / Eve. 3–7	3	28	9.33	4	69	17.25
4	Eve. 3–4 / Eve. 3–7	3	24	8	4	36	9
5	Eve. 3–4 / Eve. 3–7	2	10	5	4	36	9
6	Eve. 3–4 / Eve. 3–7	1	4	4	4	36	9
7	Eve. 3–6 / Eve. 3–9	2	16	8	5	88	17.6
8	Eve. 3–6 / Eve. 3–9	5	28	5.6	5	88	17.6
9	Eve. 3–6 / Eve. 3–9	2	0	0	5	88	17.6
10	Eve. 3–8 / Eve. 3–11	4	29	7.25	5	72	14.4
11	Eve. 3–8 / Eve. 3–11	4	29	7.25	5	72	14.4
12	Eve. 3–8 / Eve. 3–11	4	32	8	5	72	14.4
13	Eve. 3–8 / Eve. 3–11	3	21	7	5	72	14.4
	Summary	39	271	6.94	18	265	14.72

TABLE VII.

Showing that there is no progressive decrease in the proportion of male-producing females in a long-continued parthenogenetic race. Male-producing females are designated ♂ ♀, female-producing females ♀ ♀.

Generations.	No. of Young Females Isolated.	No. of ♂ ♀.	No. of ♀ ♀.	Sterile Females.	Died.	Per Cent. of ♂ ♀.	Environment.
1–144	1434	181	1188	–	65	13.22	Watch glasses. Food was miscellaneous protozoa in horse manure solution, 7–28 days old.
510 and 527	116	37	71	3	5	31.89	Battery jars. Food was miscellaneous protozoa in horse manure solution, 7–14 days old.

Chemicals.

Professor Calkins was able to stimulate his weak races of paramœcia by diabasic potassium phosphate and also by the extraction of various glands and organs of certain mammals. Different percentages of diabasic potassium phosphate, extractions of the thyroid glands of the cat and sheep, of the thymus and adrenal glands, of the pancreas, spleen and liver, of the cat, Liebig's beef extract, and alcohol were added to the culture water in the watch glasses in which the rotifers were living. Only negative results were obtained.

Death of the Parthenogenetic Race *A*, and its Proportion of Male-Producing Females.

Race *B* died out in March, 1911, in the 384th parthenogenetic generation, but race *A* was stronger and continued to reproduce parthenogenetically until June 12, 1912, when during the first hot weather the room temperature became higher than usual and this race died out in the 546th parthenogenetic generation. By its side was another unrelated race *C* in the 438th parthenogenetic generation. The latter race survived, since it was stronger, as is shown in Table I. Probably race *A* would have lived longer if the temperature had been kept lower, but eventually it would have died out, for at this time it took about four days for a generation of 4–6 young females to be produced while at the beginning of the experiments in October, 1908, it only took $1\frac{1}{2}$–2 days.

Shull states: "A progressive decrease in the proportion of male-producers with long-continued parthenogenesis occurs in some lines of *Hydatina*, perhaps in all." This may be true in some races or lines of *Hydatina*, but it does not seem to be true for all races, as Shull is inclined to believe. Table VII. shows the proportion of male-producing females in the early and in the late history of this parthenogenetic race *A*. The environment probably was more or less different in the two periods from which these data was compiled so no especial emphasis can be laid upon the higher percentage of male-producing females that occurred near the end of this race. The influence of the environment in causing male-producing females to be produced has been pre-

sented in former papers. However, the point to be noted in this table is that this race *A*, which is near the point of dying out from general exhaustion after having lived through more than 500 parthenogenetic generations, is still capable of producing a high percentage of male-producing females.

Summary and Conclusion.

These results together with those of former papers show that three races of *Hydatina senta* kept under a constant environment gradually became weaker when allowed to reproduce parthenogenetically for several years.

Two races, *B* and *A*, have finally died out in the 384th and the 546th parthenogenetic generations respectively. The other race *C* has been discontinued in the 443d parthenogenetic generation but also showed a marked lowering of vitality.

2. A wide variation in the food media including the microorganisms used as food and also the chemicals in solution did not reinvigorate the weak races. Extracts of beef, various glands of a sheep and cat, the diabasic salt potassium phosphate also were valueless as stimuli.

3. Close-fertilized eggs of the weak races subjected to periods of rest for a year in water and for eight months in the dried state, to a temperature of $-70°$ C. in water and to temperatures of $-191°$ C. and $+100°$ C. in the dried state, 6–96 hrs., failed to produce any reinvigoration.

4. The final conclusions are: that parthenogenesis can continue for several hundred generations but results in the gradual weakening and final extinction of the race; variation in the environment including food, chemicals in the food media, and temperature, do not cause a reinvigoration in such weakened races.

5. Some races show no progressive decrease in the proportion of male-producing females with long-continued parthenogenesis.

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